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EVALUATION OF PLANT GROWTH PROMOTING ABILITY OF *PROVIDENCIA* SPP. COLLECTED FROM NORTH EASTERN REGION OF INDIA IN CRUCIFERS

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ABSTRACT

The present study was conducted to evaluate the plant growth promoting ability of *Providencia* spp. collected from North Eastern Region (NER) of India in Crucifers such as Cauliflower (*Brassica oleracea* var. *botrytis* L.) and Cabbage (*Brassica oleracea* var. *capitata* L.). *Providencia* spp. namely *P. rettgeri* CIAH–3MK (JX489161), *P. stuartii* cauliflower seed–2 (KC136580) and *P. vermicola* TRB–2 (JX502189) strains originally isolated from rhizospheric soil samples of different vegetable crops grown in NER were selected for the study. These rhizobacterial strains inconsistently showed multiple plant growth promotion activities *in vitro*. *Providencia* spp. treated Crucifers seeds significantly ($p \leq 0.05$) showed highest germination percent in the laboratory than Carbendazim treated and untreated controls. Crucifers seedlings treated with all the three strains significantly ($p \leq 0.05$) increased plant growth promotion under greenhouse conditions. In both the cases of Cauliflower and Cabbage, the strain TRB–2 has showed highest modified vigour index followed by cauliflower seed–2 and then CIAH–3MK compared to Carbendazim treated and untreated controls in the greenhouse. The results are suggested that the plant growth promoting *Providencia* spp. significantly ($p \leq 0.05$) increased plant growth of Crucifers under greenhouse condition. Hence, they may be used as bio-fertilizers to promote the plant growth of Crucifers in field conditions of NER of India.

KEYWORDS: Bio-Fertilizers, *Brassica oleracea* Var. *botrytis* L., *Brassica oleracea* var. *capitata* L, Plant Growth Promoting Rhizobacteria

INTRODUCTION

North Eastern Region (NER) of India covered with dense forests has the highest rainfall in the Country with large and small river systems nesting the land (Ravindranath *et al*., 2011). Crucifers are essentially cruciferous vegetables namely Cauliflower (*Brassica oleracea* var. *botrytis* L.) and Cabbage (*Brassica oleracea* var. *capitata* L.) etc., belonging to the mustard or *Brassicaceae* (also called *Cruciferae*) family. Both are the most popular and important commercial vegetable crops grown in NER of India, particularly in the Pasighat region of Arunachal Pradesh where, the Crucifers production is heavily dependent on the seed produced in other parts of the Country, due to lack of a systematic seed production programme as well as occurrence of several seed-borne fungal diseases.

Now a day, the importance of use of PGPR has been increased worldwide and is currently used as biofertilizer for the different crop production. There are several reports available that inoculation of PGPR has significantly increased the growth and yield of Cauliflower (Solanki and Trivedi, 2013; Kushwaha *et al*., 2013; Ekinci *et al*., 2014) and Cabbage (Poonguzhali *et al*., 2008; Turan *et al*., 2014) in different agro-climatic regions of India and other Countries. Recently, the bacterial genus *Providencia* was used as PGPR inoculants for the wheat crop for the first time as they showed multiple

plant growth promoting activities *in vitro* including root colonization, nitrogen fixing activity, ACC deaminase activity, production of indole-3-acetic acid (IAA), siderophores and hydrogen cyanide (Rana *et al*., 2011; Rana *et al*., 2015).

The present study was carried out to evaluate the plant growth promoting ability of *Providencia* spp. which were originally isolated from NER of India under both laboratory and greenhouse conditions using Crucifers. Hence, the present study is proposed that, the multiple plant growth promoting ability of *Providencia* spp. may be involved in the increase of plant growth in Crucifers.

MATERIALS AND METHODS

Plant Material

Seed samples of Cauliflower (*Brassica oleracea* var. *botrytis* L.) cultivar 'Pusa Sharad' and Cabbage (*Brassica oleracea* var. *capitata* L.) cultivar 'Pusa Mukta' were collected from Indian Agricultural Research Institute (IARI), New Delhi, India. Seeds samples were surface sterilized by immersing in 1% sodium hypochlorite (NaOCl) for 30 sec and then rinsed in sterile distilled water followed by dried overnight under a sterile air stream and used in further experiments.

Collection of *Providencia* spp

In the present study, three different species of *Providencia* namely *P. rettgeri* CIAH–3MK (JX489161), *P. stuartii* cauliflower seed–2 (KC136580) and *P. vermicola* TRB–2 (JX502189) originally isolated from the rhizospheric soil samples of different vegetable crops grown in NER of India were collected from the Culture collection section, Department of Studies in Biotechnology, University of Mysore, Mysore, India and used for the study.

Characterization of *Providencia* spp. for Plant Growth Promotion Activities *In vitro*

Root colonization bioassay was carried out by following the standard procedure of Silva *et al*. (2003) using above said cultivars seeds of Cauliflower and Cabbage. Phosphate solubilization was determined by the modified method of Pikovskaya (1948), indole acetic acid (IAA) production by the modified method of Patten and Glick (2002), siderophore production by methods of Schwyn and Neilands (1987); Alexander and Zuberer (1991), hydrogen cyanide production by methods of Castric (1975); and Bakker & Shippers (1987), ACC deaminase production by Penrose and Glick (2003), chitinase production by Renwick *et al*. (1991), phytase production by the modified methods of Fiske & Subbarow (1925); and Shimizu (1992), cellulase production by the method of Cattelan *et al*. (1999) and protease production by the method of Gupta *et al*. (2002).

Evaluation of *Providencia* spp. for Plant Growth Promotion in Laboratory and Greenhouse Conditions

Twenty-five ml of bacterial suspension containing 1×10^8 cfu ml^{-1} was prepared. The surface sterilized seeds of Cauliflower and Cabbage were separately bacterized according to the method of Silva *et al*. (2003) with the bacterial suspension amended with carboxymethylcellulose (100 mg) which used as an adhesive material. Seeds treated with Carbendazim at the rate of 2 g kg^{-1} of seeds were used as a chemical treatment. Seeds soaked in sterile distilled water amended with CMC served as control. Plant growth promoting activity of *Providencia* spp. was assessed by calculating the percent germination under laboratory condition by following the top of paper method as per the standard procedure of the International Seed Testing Association (ISTA, 2005). Four replicates of 100 seeds were carried out for each treatment and the experiment was repeated three times.

The greenhouse experiment was carried out at the Department of Studies in Biotechnology, University of Mysore, Mysore, India. The potting medium containing the soil: sand: farm yard manure @ 2:1:1 (v/v/v) was autoclaved for 1 h at 121°C on two consecutive days. The sterilized potting medium was filling into 12 cm diameter plastic pots. Bacterized, Carbendazim treated and untreated seeds of both Cauliflower and Cabbage were sown separately in the plastic pots. The Crucifers seedlings were grown in a greenhouse under a day/night cycle of 16/8 h and at the temperature of 28/20°C with around 80% of relative humidity. Watering was done regularly. After 30 days of sowing, the seedlings were sampled and analyzed for plant growth parameters as per the standard procedure of the International Seed Testing Association (ISTA, 2005). The plant growth parameters such as percent germination, shoot length, root length, fresh weight and dry weight were measured. The modified seedling vigour index was calculated using the following formula as described by Abdul Baki and Anderson (1973). Modified Vigour Index = [(Mean root length + Mean shoot length) × (% of germination)] × Dry weight. Ten plastic pots were maintained for each treatment for each crop and arranged in a completely randomized block design (RCBD) in a greenhouse with three replications. The experiment was repeated three times.

RESULTS

Characterization of *Providencia* spp. for Plant Growth Promotion Activities

All the three *Providencia* spp. were showed inconsistently varied results for the plant growth promotion activities in laboratory. All these strains were colonized the roots of Cauliflower and Cabbage and produced indole acetic acid (IAA), ACC deaminase and protease enzymes. In addition, the strains CIAH–3MK and TRB–2 were also solubilized the phosphate and produced siderophore, hydrogen cyanide and phytase enzyme, but not produced the chitinase and cellulase enzymes. Apart from these the strain cauliflower seed–2 was having the ability to produce the chitinase and cellulase enzymes but could not solubilize the phosphate and produce siderophore, hydrogen cyanide and phytase enzyme.

Evaluation of *Providencia* spp. for Plant Growth Promotion in Laboratory and Greenhouse Conditions

The laboratory experimental data revealed that all the three *Providencia* spp. were significantly ($p \leq 0.05$) showed higher percent of germination than the Carbendazim treated and untreated controls in both Cauliflower and Cabbage. In case of Cauliflower, the strain TRB–2 showed significantly ($p \leq 0.05$) higher % of germination (91) followed by cauliflower seed–2 and CIAH–3MK with the germination of 90 and 89% respectively. The Carbendazim treated and untreated controls had the germination of 85 and 79% respectively (Figure 1). Where as in case of Cabbage, the strain TRB–2 also showed significantly ($p \leq 0.05$) higher germination of 90%. This was also followed by cauliflower seed–2 and CIAH–3MK with the germination of 88 and 87 respectively. The Carbendazim treated and untreated controls had the germination of 84 and 78% respectively (Figure 1).

The greenhouse experimental data revealed that all the three *Providencia* spp. were significantly ($p \leq 0.05$) showed higher plant modified vigour index (MVI) than the Carbendazim treated and untreated controls in both Cauliflower and Cabbage. The results found in greenhouse that the strain TRB–2 showed significantly ($p \leq 0.05$) higher MVI of 6206 followed by cauliflower seed–2 and CIAH–3MK with the MVI of 5590 and 5200 respectively in case of Cauliflower. The Carbendazim treated and untreated controls had the MVI of 4334 and 1680 respectively (Figure 2). While in case of Cabbage, the strain TRB–2 also showed significantly ($p \leq 0.05$) higher MVI of 5940 followed by cauliflower seed–2 and CIAH–3MK with the MVI of 5380.5 and 4998 respectively. The Carbendazim treated and un-treated controls had the MVI of 4085 and 1737.5 respectively (Figure 2).

DISCUSSIONS

In the present study three different *Providencia* spp. namely *P. rettgeri* CIAH–3MK, *P. stuartii* cauliflower seed–2 and *P. vermicola* TRB–2 strains which were originally isolated from the rhizospheric soil samples of different vegetable crops grown in NER of India were collected, characterized and evaluated for the plant growth promotion of Crucifers such as Cauliflower and Cabbage under both laboratory and greenhouse conditions. In our study also all the three *Providencia* spp. were inconsistently showed varied results for the plant growth promoting activities *in vitro*. For the first time, Rana *et al*. (2011) reported the genus *Providencia* which showed multiple plant growth promoting potentials as PGPR inoculants for the wheat crop. The multiple plant growth promoting potentials showed by *Providencia* was included wheat root colonization, nitrogen fixing potential, ACC deaminase activity, production of IAA, siderophores and hydrogen cyanide.

The laboratory data showed that *Providencia* spp. were significantly ($p \leq 0.05\%$) higher germination percent than the Carbendazim treated and untreated controls in both Cauliflower and Cabbage. The greenhouse experimental results revealed that all the three *Providencia* spp. were significantly ($p \leq 0.05\%$) showed higher plant modified vigour index than the Carbendazim treated and untreated controls in both Cauliflower and Cabbage. In both crops, these three strains were considered as the most potential PGPR strains as they had multiple plant growth promoting ability. Among these strains, *P. vermicola* was the most potential than other two *Providencia* spp. *viz*., namely *P. stuartii* and *P. rettgeri*. The root colonizing, phosphate solubilizing, IAA, siderophores, hydrogen cyanide and protease producing and ACC deaminase activity of *Providencia* spp. are the main plant growth promoting activities which contributed to promote the plant growth promotion in Crucifers.

Solanki and Trivedi (2013) reported that the Cauliflower plant vigour index was the higher in PGPRs treated seeds as compared to fungicide and control treatments. Recently, Ekinci *et al*. (2014) also determined the effect of different PGPR strains on growth and quality of Cauliflower transplants under greenhouse conditions and reported that the different bacterial inoculations increased plant growth parameters of Cauliflower transplant. PGPR treatments also improved the seedling growth and quality in Cabbages compared with the control (Turan *et al*., 2014). Seed bacterization with Phosphate solubilizing bacterial (PSB) strains increased the root elongation and biomass of Chinese cabbage and the plant growth promotion by them could be due to the production of phytohormones or mechanisms other than phosphate solubilization (Poonguzhali, *et al*., 2008). Rana *et al*. (2015) also reported that inoculation with *Providencia* sp. PW5 was recorded the highest yield of 5.23 Mg ha^{-1} and increased the grain yield by 44.62% over the fertilizer control in wheat.

CONCLUSIONS

In conclusion, *Providencia* spp. used in the present study were considered as the most potential PGPR strains used as biofertilizer to promote the plant growth in Crucifers. Kaushal *et al*. (2012) suggested that the use of PGPR as biofertilizer was beneficial for Cauliflower cultivation in Himachal Pradesh as it enhanced growth of Cauliflower. Further work has to carry out to demonstrate the application of talc-based formulation of *Providencia* spp. as seed bacterization and soil application for the promotion of Cauliflower and Cabbage in field conditions of NER of India.

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APPENDICES

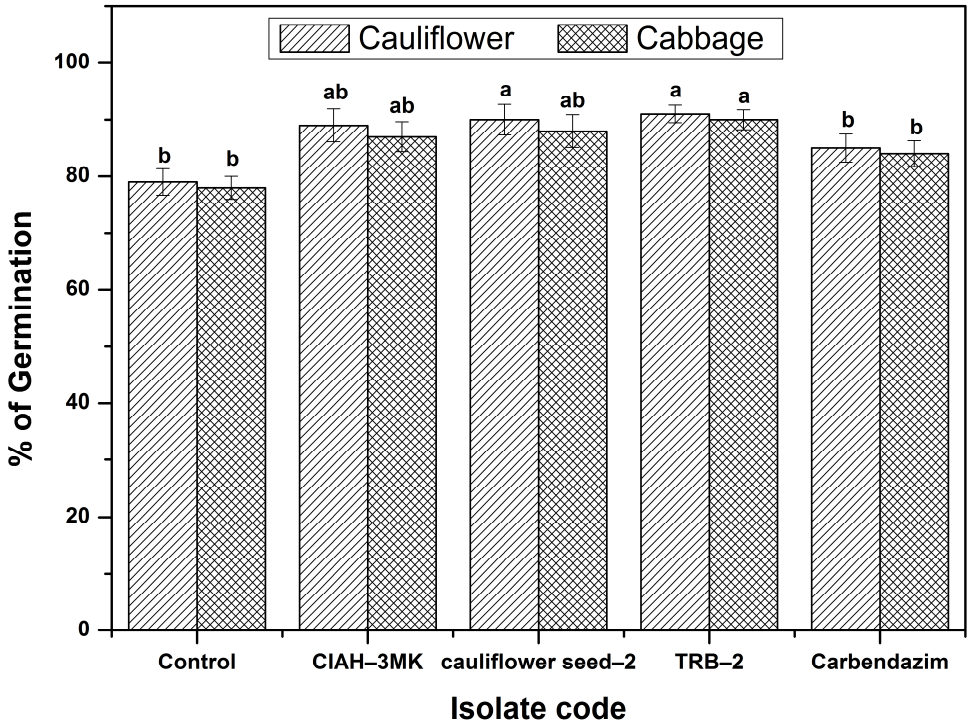

Figure 1: Plant Growth Promotion Activities of *Providencia* spp. in Crucifers in Laboratory. Values are Means of Three Replications. Values with the Same Letter are not Significantly Different at $p \leq 0.05$

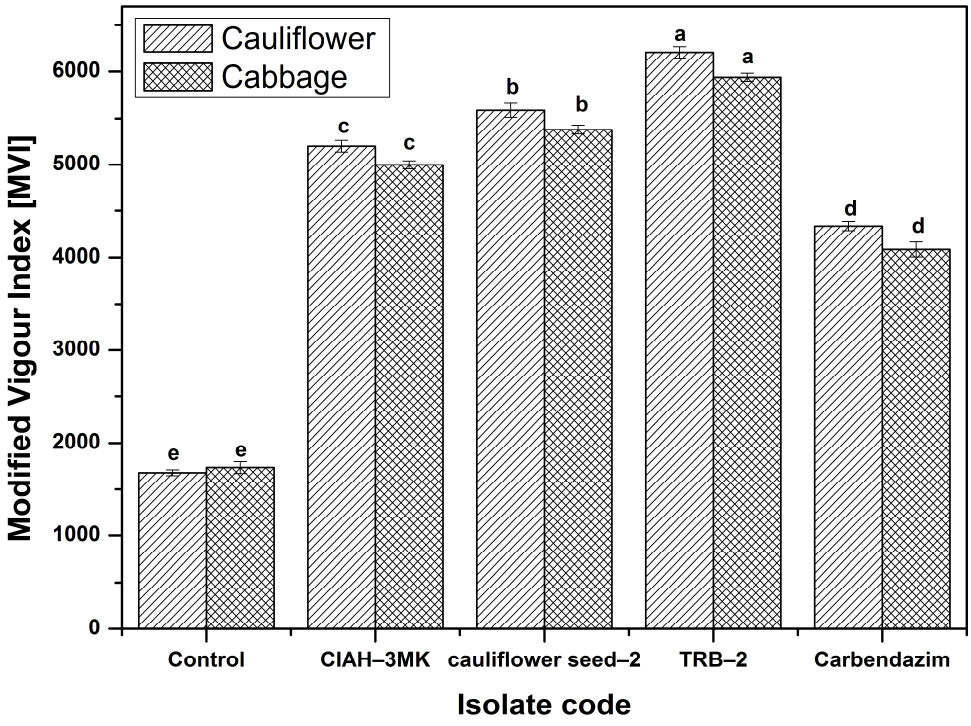

Figure 2: Plant Growth Promotion Activities of *Providencia* spp. in Crucifers in Greenhouse. Values are Means of Three Replications. Values with the Same Letter are not Significantly Different at $p \leq 0.05$